MONTANA DEPARTMENT OF AGRICULTURE

ENVIRONMENTAL MANAGEMENT DIVISION

Current Literature Review

on 2, 4, 5-T

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This paper is a review of the scientific literature currently available on 2,4,5-T. The Montana Department of Agriculture, Environmental Management Division, will maintain this information on file. As data from on-going or future studies are reported, conclusions stated herein may be supported or negated.

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HISTORY OF REGULATION

Since its registration in the mid-1940's, production and use of 2,4,5-T in the United States increased rapidly, with production reaching a peak in 1968 due to military purchases. Production has gradually declined, more rapidly over the last couple of years.

The Vietnam war brought about a significant amount of criticism to the chemical Agent Orange, a 50-50 mixture of n-butyl esters of 2,4-D and 2,4,5-T used to defoliate the dense jungle vegetation to prevent ambushes by the enemy. The criticism led to investigations by the National Cancer Institute in 1964 into the possible birth defects and carcinogenic potential of the herbicides. Although the results from the tests proved positive in susceptible species given massive doses, the report was kept confidential to avoid alarming the public with results from tests involving unrealistic conditions.

Public disclosure of the information initiated congressional hearings in April on "The Effects of 2,4,5-T on Man and the Environment".

On April 15, 1970, the HEW and USDA issued a joint order suspending all uses in lakes, ponds or on ditch banks and liquid formulations used around the home, recreation areas and similar sites. The order stopped 2,4,5-T use immediately since it was considered to be an imminent hazard.

The same two departments issued another order on May 1, 1970 cancelling all 2,4,5-T uses on food crops intended for human consumption and granular formulations used around the home, recreation areas and similar sites. This cancellation was appealed by two manufacturers of 2,4,5-T who wished to continue 2,4,5-T use on rice. According to Federal Regulations (Federal Insecticide, Fungicide and Rodenticide Act) the appeal allows for continued use of the product until the issues are resolved. An Advisory Committee was formed in late 1970 to investigate research and hear testimony on 2,4,5-T.

In May 1971, the Advisory Committee submitted its recommendations to the Environmental Protection Agency (EPA) which had been newly formed and given the authority over pesticide matters. The committee recommended that all uses of 2,4,5-T registered prior to April of 1970 be reinstated, with the provisions of establishing permissible residues in foods and that the TCDD content in 2,4,5-T be kept to low levels.

The Administrator of EPA rejected the committee's recommendations and called for a public hearing in August of 1971 on all uses of 2,4,5-T. The hearings were delayed until June of 1974, when the EPA announced that it was cancelling the hearings and withdrawing only the cancellation of 2,4,5-T use on rice.

All earlier suspensions of uses remained in effect as did all cancellation of uses of 2,4,5-T for which there was no appeal or request of a hearing. The hearings were cancelled as no evidence was available to link 2,4,5-T use to

carcinogenic and teratogenic effects in humans. EPA continued its investigations, especially in the dioxin area.

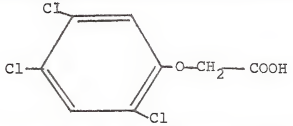
Nothing new developed until April 11, 1978, when the EPA issued a notice of Rebuttable Presumption Against Registration (RPAR) for all pesticide products containing 2,4,5-T (43 FR 17116, April 21, 1978). RPAR initiates a comprehensive public review of all 2,4,5-T registrations and all pending applications for registrations of 2,4,5-T products.

On February 25, 1979, the EPA ordered emergency suspension of and issued notices of intent to cancel the use of 2,4,5-T on forests, rights-of-way and pastures. The RPAR review continued for those uses which were not subject to the emergency suspension orders. Non-suspended uses were rangeland and non-crop uses. Notices of intent to hold a hearing on whether or not to cancel these non-suspended uses were issued by EPA on December 3, 1979.

The RPAR review for non-suspended uses is still going on. At the present time, 2,4,5-T can be used on rangeland and also for rice and non-crop uses. See Federal Register on December 13, 1979, 72316 for detailed definition of non-crop uses. Because the EPA issued an emergence suspension for forests, rights-of-way and pasture uses, continued use is not allowed at any time. The hearing will determine whether to reinstate these uses or to cancel them.

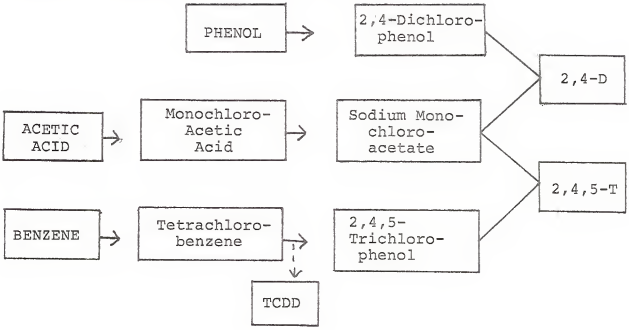
PHYSICAL AND CHEMICAL PROPERTIES

2,4,5-T is in the family of Chlorinated Phenoxyalkanic Acids. General use is for the control of woody and herbaceous plants. It exists as an acid but numerous commercial concentrates are available in amine salt or ester formulations. Specific chemical and physical properties of the acid can be found in the Herbicide Handbook of the Weed Science Society of America. The molecular formula for 2,4,5-T is: $C_8H_5Cl_3O_3$.



The usual procedure for the synthesis of 2,4,5-T acid involves the reaction of 2,4,5-Trichlorophenol with sodium mono-chloracetate in an alkaline aqueous medium. The esters and amines can then be produced from the free acid. The phenoxy herbicides readily form salts with alkali metal ions, ammonia and amines (NRCC, 1978). The manufacturing of 2,4,5-T is similar to that of 2,4-D except that 2,4,5-Trichlorophenol is needed as a starting material for 2,4,5-T. The synthesis of the trichlorophenol is a sensitive process. The temperature of the reaction is crucial and must be maintained at 160°C. If the temperature goes above this, the toxic impurity of 2,4,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is formed (see Figure 1). TCDD is not a contaminant of all phenoxy acids.

Figure 1. Synthesis of 2,4-D and 2,4,5-T



Formulations

2,4,5-T is a weak acid that is slightly soluble in water and petroleum oil. The acid is the active form and is often converted to water-soluble amines or oil-soluble esters for commercial use. These formulations are then mixed with other agents depending on the intended use. These agents include thickeners, emulsifiers, solvents and wetting agents.

The esters normally exhibit greater herbicidal activity than the parent acids due to improved absorption by the target plants. Esters are more volatile than salts and may be a hazard to non-target crops. The problem of drift has been minimized by the introduction of low-volatile esters. The specific herbicical activity of 2,4,5-T will be discussed further in Biological Activity.

Contaminants

It is well known that 2,4,5-T is contaminated with TCDD, a very toxic dioxin. Most commercial products do contain small amounts of TCDD with present regulations limiting that amount to less than 0.1 mg/kg (Stalker, cited in NRCC, 1978). The mechanism of the systemic toxic action of TCDD is relatively unknown. The chemical structure may be a factor of its potent toxicity (Gribble, 1974).

The toxic effects of TCDD on laboratory animals have not been agreed on by researchers though most agree that TCDD is a teratogen. The results of carcinogenic and mutagenic studies are variable. No causal relationship has been revealed linking TCDD to cancer in humans, but it is suspected of being carcinogenic in laboratory animals (U.S. EPA, 1979; Scientific Dispute Resolution Conference, 1979; Gehring and Betso, 1978). Existing reports on the mutagenicity of TCDD are not definitive (Moore, 1978).

BIOLOGICAL ACTIVITY

Uptake

Phenoxy acids can be absorbed by plant foliage, roots and soft stem tissue (CAST, 1978).

Absorption into the roots occurs in a few plant species when the herbicide is applied to the soil. The mechanism of movement seems to involve absorption by root hairs and cortex parenchyma in the primary region behind the root tip. From here, the herbicides move from the symplast where they leak into the apoplast and ascend into the foilage via the transpiration stream of the xylem (Ashton and Crafts, 1973).

Corbin et al. (1971) have shown that soil pH has profound effects on the uptake of herbicides by roots. Absorption is promoted by a low external pH. Once absorbed by the roots, movement is somewhat restricted. The herbicide may be accumulated within the symplast to higher concentrations than the external medium.

More commonly, the herbicide is applied to the foliage. Foliar uptake is essentially a two step process. The first step is entry into the plant. The second step involves movement through the plant tissues into the internal medium. To get into the leaf, the herbicide must cross the waxy cuticle, which presents an effective barrier against some herbicide formulations. Ammonium and amine salts of the phenoxy acids penetrate the cuticle more readily than the sodium and potassium salts. Lower pH of the applications medium allows the

free acid to move quickly past the barrier. Esters also readily penetrate the cuticle but short-chain alkyl esters do not translocate as easily as esters made from long-chain alcohols (Loos, 1975).

Adding surface-active compounds as emulsifying agents may increase toxicity by increasing the amount absorbed. Surface-active compounds help dissolve surface waxes and may loosen the structure of the cuticle. Inorganic ions seem to work best as an emulsifier.

An increase in temperatures also promotes increased absorption and subsequent injury to the plant. High relative humidities stimulate the absorption and translocation whereas moisture stress in a plant reduces uptake and movement.

Absorption could also take place through the stomata, although conflicting reports make it difficult to evaluate the importance of this alternate route. Some workers have noted that uptake through the stomata is possible but relatively unimportant. Spraying with phenoxy herbicides usually results in closed stomata and reduced transpiration (Brian, cited in Aberg and Eliasson, 1978). More field work is needed concerning active absorption through plant stomata.

Application via the stem is also possible with phenoxy herbicides. The process is similar to foliar absorption in green or succulent stems. Stems covered by bark require an oil carrier when applied as a basal spray. An aqueous solution can be used if a cut is made in the bark first.

For a more detailed explanation of herbicide absorption,

please refer to Mode of Action of Herbicides by Ashton and Crafts.

Translocation

Once past the cuticle barrier, the herbicide can move through the plant by either the symplast, including the sieve-tube system of the phloem, or the non-living apoplast, which includes the cell-wall system and the conducting elements of the xylem. Movement with the food supply (phloem) is generally found by researchers.

There is much evidence to support the movement of herbicides from regions of photosynthesis to regions of active growth, where the food supply is needed. So, active movement of foods in the plant is a requirement for thorough distribution of the herbicide. In mature plants, the phloem-mobile herbicide applied to the upper foliage moves into the shoot tips, flowers and fruits, while the molecules applied to lower leaves move into the roots. Those mature leaves that are contributing to the food supply are passed by. Transport in seedlings is into the roots. The movement of the herbicide from xylem to phloem and vis versa is possible although not common (NRCC, 1978).

The movement of 2,4-D and 2,4,5-T in the phloem is restricted compared with other phenoxy herbicides. In the phloem, the less mobile herbicide compounds tend to accumulate in the parenchyma and move out of the mainstream. As a result, the concentration of 2,4-D and 2,4,5-T in the phloem is constantly diminishing, reducing the desired herbicide effect on the

plant (Loos, 1975). The more rapid the translocation process, due to increased food movement, the less likely the herbicide will accumulate in the cells. Weed control is best achieved when the plant is undergoing vigorous root growth, with the food moving rapidly down to the growing roots, carrying along with it, the herbicide.

An accumulation problem also appears when these herbicides are applied to the roots. They may become bound to living cells in the roots. Root translocation can be a benefit to less phloem-mobile herbicides since movement from roots is via the xylem. When dosage is increased sufficient to produce strong contact action, the herbicides penetrate and move rapidly in the transpiration stream resulting in rapid death of the plant.

Mode of Action

2,4,5-T belongs to the large group of plant growth-regulators designated as auxins. Auxin is an organic substance which, at low concentrations (less than 10^{-3}M) promotes growth along the longitudinal axis of shoots and inhibits elongation of roots (Loos, 1975). Phenoxy acids are synthetic auxins though, and do not always have the same effects on a plant as natural auxins do. Normal control of growth is lost in the case of herbicides, as they do not respond to those mechanisms that control natural auxin concentrations. Phenoxy herbicides can also behave as antiauxins, which prevent auxins from reacting in the manner needed to trigger growth response in the plant.

Synthetic auxins stimulate nucleic acid and protein synthesis which leads to a further stimulation of cell growth. This growth can be abnormal. The death of the plant begins with the suppression of normal apical growth along with the initiation of abnormal axis growth. Robertson and Kirkwood (cited in NRCC, 1978) reviewed the research concerning mode of action and concluded that action of phenoxy herbicides involves a complex series of reactions that begin with the depression of the gene regulating syntheses of the enzyme RNAase. This results in the synthesis of RNA and protein which in turn, brings about "massive cell proliferation". The proliferation, which usually occurs in the stem, leads to the disruption of the transpiration and translocation systems, resulting in the accumulation of assimilate in the shoots and starvation of the roots. The vascular tissues may become so plugged as to result in lack of nutrients to areas in the plant normally supplied via these tissues. The ability to absorb water and salts is affected by the herbicide as well as inhibition of photosynthesis.

A summary of generalized effects in susceptible seedlings are: normal growth pattern changes rapidly, meristematic cells cease dividing, elongating cells stop length growth but continue radial growth. In mature plant parts, parenchyma cells swell and divide, root elongation stops and root tips swell while young leaves stop expanding and develop excessive vascular tissues. The herbicides are selective and noticeable effects on plants will vary with the species

treated and the phenoxy herbicides used.

The selectivity of the phenoxy herbicide varies in nature. Internally, a plant may have a successful means of detoxifying the compounds, by altering the mode of action process. Difference in structural features of two plants may render one susceptible, the other not. For example, the effectiveness of phenoxy acids on dicotyledonous and not on monocotyledonous plants could be attributed to the phloem structure. Non-affected monocots have their phloem scattered in bundles, surrounded with protective tissue unlike dicots. Also, movement of the herbicide within the plant is more restricted in monocots than dicots (Loos, 1975).

Among dicotyledons, resistance is determined by not only the rate or amount of herbicide uptake and translocation, but also the metabolism and excretion of the herbicide in the plant. Excretion from the roots could be a possible detoxification reaction from some resistant species. Plants which rapidly degrade or bind (conjugate) the phenoxy acid are successful against the actions of the herbicides. Binding the chemical with protein in wheat plants render the herbicide non-toxic (NCRR, 1978). Plants may actively metabolize the parent compound to nonherbicidal degradation products. The ability alone of the plant to degrade or conjugate active phenoxy herbicide does not make the plant resistant. The speed of the detoxification process is an important factor.

BEHAVIOR IN WATER

2,4,5-T can enter the surface water through direct application, runoff, drift, or leaching.

Leaching has not been considered important as a means of transporting 2,4,5-T into bodies of water. Although 2,4,5-T is mobile in soil, much of the herbicide is degraded quickly, reducing the possibility of leaching into water supplies.

Lawson (1976) studied 2,4,5-T residues in storm run-off from small upland watersheds which were sprayed once a year for three consecutive years. During the first spray period a storm three weeks after spraying produced an average of 2.1 ppm on cleared area and 1.0 ppm on partially cut area. The second and third applications did not result in detectable residues in storm runoff. No herbicide was detected in the nearby stream. He concludes that 2,4,5-T is rapidly degraded in streams.

Reigner et al. (1968) treated vegetation along two stream banks with normal amounts of acid and ester formulations of 2,4,5-T. Samples of the water were analyzed for three weeks, including odor tests. Results indicated no adverse effects on the streams if applied with normal precautions. 2,4,5-T could be used on municipal water sheds without any water contamination.

Photodegradation is an important means to eliminate 2,4,5-T residues in water. Crosby and Wong (1973) see sun-

light degradation as significant and hastens the elimination of residue. TCDD was not found to be a major product of photodetoxification. Photolysis of 2,4,5-T is slow however.

Microbial degradation in the water occurs mainly in warm, aerobic streams and rivers where a relatively large population of microorganisms may be found. Once the 2,4,5-T reaches the bottom sediment, residues may persist longer there (Advisory Committee, 1971).

Persistence/Residues

Once in the water, 2,4,5-T does not persist for any length of time, especially if correct rates of application are used. Rapid dilution with downstream movement can effectively reduce concentrations of 2,4,5-T in streams and rivers (Montgomery and Norris, 1970). A point downstream can be reached where no detectable residues occur.

In a two year survey of major rivers and streams in the Western U.S., the U.S. Geological Survey found herbicide concentrations, including 2,4,5-T, were never in excess of the permissible limits established for public drinking water. 2,4-D was the most commonly detected herbicide (Manigold and Schulze, 1969). Waldron (cited in NRCC, 1978) reported no residues in water and seidment samples collected monthly during a one year period. Samples were taken from five river systems in Ohio following normal use of the herbicide.

In a stream which was completely in the sprayed acreage, Norris (1967) found 2,4,5-T residues for 16 weeks after treatment.

Ontario wells and farm ponds were investigated for phenoxy herbicide contamination and the results found 32% of the wells contained detectable 2,4,5-T residues. Some of the cases were due to direct contamination by some means.

BEHAVIOR IN SOILS

The ability and rate of absorption of the herbicide into the soil will, for the most part, determine the relative persistence of the chemical in the soil. The quality and type of clay and organic matter, the soil structure, water content, temperature and pH all affect the distribution and susceptibility to degradation of the herbicide. The formulation of the herbicide does not seem to make any difference in the absorption of the herbicide because the various esters, amines and other salts dissociate or hydrolyze in the case of esters, leaving the parent acid compound.

Leaching of the herbicide is limited by the degree of absorption into the soil components. In relatively acidic soils, ones with high organic components, leaching is restricted (Torstensson, 1978; Norris et al., 1977). CAST (1975) agrees that the herbicide is bound to soil particles in the upper part of the soil. They do not leach into water tables and contaminate wells and springs. CAST also states that movement into streams from surface runoff is not significant. Mobility of the herbicide is thought to increase with the addition of surfactants (NRCC, 1978). Helling (cited in NRCC, 1978) has determined that phenoxy acid herbicides are more mobile in soils than generally believed. Norris et al. (1977) determined that 2,4,5-T sprayed in an Oregon forest had limited mobility and leaching power due to strong absorption into the forest litter.

The possibility of 2,4,5-T getting into the ground water supplies in any significant amount is remote even with high doses of the herbicide (NRCC, 1978). Residues of 2,4,5-T tend to remain in upper soil layers.

Degradation in the soil can occur by biological, non-biological or a combination of both systems. As mentioned above, the esters of 2,4,5-T undergo a chemical reaction (hydrolysis) before the herbicide is biologically degraded. Other chemical reactions include oxidation and reduction. Photodecomposition is also thought to occur. This involves decomposition by natural sunlight and can happen only at the soil surfaces. Absorption of the herbicide into the soil limits the effects of photodegradation. Although much work has been done to help explain photodecomposition, most of it has been done under artificial conditions using UV light in laboratories. The influence of light and its affects on the phenoxy acids in soils is not yet clear in the natural environment.

It is generally accepted that degradation in the soil of phenoxy herbicides involves the microbial population of the soil. Scientific views are somewhat split on the issue of microbial degradation of 2,4,5-T. Some researchers report that 2,4,5-T is resistant to microbial degradation while others support the phenomenon.

Important to microbial breakdown of the herbicide is the amount of organic content in the soil. Soil samples that had been sterilized by autoclaving do not degrade phenoxy

acids as quickly as untreated soils, suggesting the active role of soil microorganisms. Moist, warm soils favor high microbial populations.

The mechanics of degradation of phenoxy herbicides in the soil by microorganisms has been reviewed by the Canadian Research Council of Canada (NRCC, 1978). When the herbicide reaches the soil, there appears to be an initial period when degradation is minimal, if any. This is followed by the rapid degradation of the herbicies (Audus, 1960). This lag period in which no apparent action is taking place is characterized as a period of growth and adaptation for the soil microbial population. These soil microorganisms are unable to metabolize many of the herbicides immediately. The delay before degradation starts is the time required to form the enzymes that catalyze the decomposition of the chemical. The potential of soil organisms to degrade a phenoxy herbicide depends on whether or not they possess an enzyme system that normally metabolizes compounds related to the herbicide. If so, the enzyme can be formed from the closely related enzyme already present (Mullison, 1970). Once the readied microorganism begins to increase, degradation begins and accelerates according to exponential growth. The resulting microorganisms are said to be "enriched" and will retain their ability to degrade the herbicide for as long as a year between applications and will eliminate or significantly shorten the lag period before degradation (NRCC, 1978).

Reigner et al. (1968) indicated that decomposition of

2,4,5-T results in CO_2, inorganic chlorides and water with chlorophenols not being detected as an end product.

Norris et al. (1977) investigated the behavior of 2,4,5-T in soils and on the vegetation of a Northwest forest. Two aerial applications were made. Minimum detectable residue was 0.01 ppm. 2,4,5-T is rapidly absorbed in forest floor litter. Residue levels on the forest floor declined linearly with time after the application suggesting first order kinetic rate of disappearance. Extremely low levels in the soil demonstrates the rapid absorptive ability of the forest floor material. The herbicide, once absorbed, degrades rapidly in the organic material indicating abundant microbial activity into the soil. Microbial degradation of 2,4,5-T is also supported by Montgomery and Norris (1970).

Smith (1978) studied the persistence of 2,4,5-T in Saskatchewan soils and found no evidence to support the hypothesis that tri-chloro herbicides were more resistant to microbiological degradation than the di-chloro herbicides. Results indicated that in all soils at 20°C. and 85% of field capacity moisture, 2,4,5-T underwent rapid breakdown. There was a considerable decrease in the amount of breakdown found in air-dried soils which supported low, if any, microbial populations.

Those researchers who hold that 2,4,5-T is resistant to microbial breakdown do so on the basis of chemical structure of the herbicide. The adding of one more chloride in the structure of the compound makes the herbicide less available

to microbial breakdown in the soil (Leopold et al., cited in U.S. EPA, 1979). Also, compounds that have a chlorine in the meta position of the aromatic nucleus, like 2,4,5-T structure, are limited in their transformation to any appreciable extent (Torstensson, 1978).

The difference in these two cases of thought may be explained by the lack of microbial growth during degradation of 2,4,5-T (Torstensson, 1978). This growth is a characteristic of microorganisms degradation as mentioned earlier. Those chemical compounds that do not sustain microbial growth are thought to undergo degradation by means of co-metabolism.

In summary, complete degradation of 2,4,5-T can be via a series of co-metabolic reactions, or by the action of microbial species. Overall, the phenoxy herbicides will not make soil sterile or permanently infertile (Mullison, 1980; Torstensson, 1978).

Persistence/Residues

The length of persistence in the soil varies, but it does not usually carry over from one growing season to the next (Montgomery and Norris, 1970; Sheets and Harris, 1965; CAST, 1975). The question of possible residues from spraying last year or the year before is a concern to growers using the chemicals. The concern is whether or not sensitive crops will be affected. Sheets and Harris (1965) studied the topic of crop rotation and the effects of herbicide residues. They found that herbicides which injure plants longer than three or four months after application at normal rates can be

considered a potential hazard to crops grown in rotation. Furthermore, herbicides that exhibit a lag phase before microbial breakdown are usually not hazardous to subsequent crops. For maximum protection of crops, spraying early is best. The later the spraying, the greater the chance of residue carrying over to the next year.

Smith (1978) calculated the half-lives of 2,4,5-T in heavy clay, clay loam and sandy loam soil types to be 3, 13 and 10 days respectively. This was twice the length of time found for 2,4-D half-lives in the same soil types. DeRose and Newman (cited in EPA, 1979) state 2,4,5-T persists two to three times longer than 2,4-D. Newman et al. (cited in NRCC 1978) determined the 2,4,5-T half-life under field conditions to be 19 weeks. Estimates range from 24 hours to 9 months (Montgomery and Norris, 1970). Such factors as rate of application, condition of the soil and time of year all add to the variance of persistence of 2,4,5-T in the soil. Most researchers agree that 2,4,5-T persists longer than 2,4-D.

RESIDUES - ANIMAL TISSUES, MILK AND CREAM

The greatest potential exposure of livestock to herbicides is from grazing areas that have been chemically treated and, to a lesser extent, from a diet of contaminated feed. The question of great concern to the public is: will exposure cause a buildup of residues in meat, milk and cream? If residues are found in tissue or milk samples, this could be evidence for bio-accumulation of herbicides in animals. Today most researchers agree that due to rapid excretion from mammals and overall degradation in the environment, phenoxy herbicides, including 2,4,5-T, do not bio-accumulate or move up the food chain (HALTS, 1980; MDH, 1978; Gehring and Betso, 1978; Mullison, 1970; NRCC, 1978; Leng, 1972; Montgomery and Norris, 1970).

Bjerke et al. (1972) fed cows a complete ration containing 2,4,5-T at six levels from 10 to 1000 ppm for two or three weeks at each level. No residues of 2,4,5-T greater than 0.5 ppm were found at the 10 to 300 ppm feeding levels. At the 1000 ppm feeding level, average residues found were 0.42 ppm in milk and 0.26 ppm in cream. Withdrawal from treated feed brought about a rapid disappearance of residues from the milk. All residues then decreased below 0.05 ppm by the third day after withdrawal from 2,4,5-T treated feed. It was noted that residues increased as chemical feeding rates increased, but all feeding rate residues reached a plateau level on the second or third day of sampling.

Similar results were found in milk and cream samples taken during a feeding study done by Leng (1972). After a diet of 10 to 2000 ppm of 2,4,5-T for four weeks, 1300 milk and cream samples were analyzed for 2,4,5-T residues. Samples were also taken from cows given one week of untreated feed following the four weeks of treated feed. No residues(>0.05 ppm) occurred in milk or cream of cows given 10 to 300 ppm of 2,4,5-T. Dosage levels above 300 ppm resulted in some residues. In all cases, levels of residues decreased rapidly following a one week diet of untreated feed, to a level below 0.05 ppm.

Leng also analyzed 1400 meat tissue samples for 2,4,5-T residues. Some residues were detected in muscle, fat and liver tissues (0.12 to 0.28 ppm) and also in kidney samples (3.3 ppm). Again, residue levels increased with the dose level. Leng concludes that 2,4,5-T and its phenol are not likely to be found in commercial food items such as meat, fat or meat by-products of livestock grazing in pastures and rangeland treated with 2,4,5-T. There is not evidence that phenoxy herbicides are found in bound or conjugated form in animal tissues.

2,4,5-T residues found in tissues of sheep fed 2000 ppm for 28 days averaged 1.0 ppm in muscle and 0.25 ppm in fat for those animals killed on the same day as the final dose. Tissue samples collected from animals that were held for a week on untreated feed containted residues which did not exceed 0.05 ppm. Residues ranged from 0.5 to 5.5 ppm in liver and from 5.0 to 7.0 ppm in the kidney tissues of the

nonwithdrawal group. High residue levels in the kidneys are expected, as the major route of elimination of phenoxy herbicides is by excretion via the urine. After withdrawal for seven days, kidney and liver residues were down to less than 0.05 ppm (Clark et al., 1975)

The forage which the livestock graze is not expected to be a toxic source of exposure if the herbicide is applied at normal rates used in agricultural practice. Maximum residues are likely to occur when sprays are applied directly to grass. Normal applications would produce lower residues. Leng (1972) estimates that residues on forage are not likely to exceed 300 ppm immediately after treatment and will decline to less than 100 ppm within two weeks after treatment, if used at rates of two to three pounds per acre.

Bovey and Baur (cited in U.S. EPA, 1979) investigated the persistence of 2,4,5-T on assorted forage grasses in Texas. The study areas varied in soil type and climate. An average of 98% of the 2,4,5-T was lost from all treated areas six weeks after treatment. After 26 weeks, levels in the grass ranged from 0 to 51 ppm.

Persistence of 2,4,5-T on range forage grasses is mainly influenced by the amount of rainfall. Formulation had no significant affect as esters hydrolyzed to the acid in a short period of time on the leaf surface (Morton et al., 1967).

Keeping livestock off the treated forage for a few weeks and also delaying the slaughter of livestock which feed in

treated fields will help to minimize any residues which may be present. Instructions for grazing can usually be found on the 2,4,5-T product label.

ABSORPTION, DISTRIBUTION AND EXCRETION IN MAMMALS

Phenoxy herbicides are absorbed from the gastrointestinal tract and become widely distributed in the tissues (Paulson 1975). More than 80-85% of an ingested dose is assimilated by mammals under most conditions. Assimilation does not appear to be related to the magnitude of the dose (NRCC, 1978).

Following absorption from the gastrointestinal tract, the herbicide is reversibly bound to plasma proteins which results in their retention in the plasma and/or extracellular compartments (Gehring and Betso, 1978). Studies indicate that the concentration of herbicide in the plasma increases rapidly after administration of the dose and reaches a peak at which point the plasma concentration declines steadily, apparently at a first-order rate of decrease (Gehring et al., 1973; Khanna and Kohli, 1975). The residues found in tissues are thought to decline at the same rate as residues in the plasma (NRCC, 1978).

The major route of elimination is via the urine, primarily as the parent compound with small amounts of the 2,4,5-T becoming conjugated before excretion. This is especially true after administration of high doses. The tissue and plasma half-lives are consistent in laboratory animals and humans, although some variation naturally occurs due to the individual's body chemistry. The urinary excretion of phenoxy herbicides occur by an active process which could be overwhelmed when a large dose is given. At this point,

not only does the half-life increase, but fecal excretion, tissue distribution and metabolism of the compound increases to compensate for the increased load (Gehring and Betso, 1978). This does not mean that the herbicide accumulates in the body. When such a large dose is given that it overwhelms the urinary system, it becomes toxic to the mammal producing noticeable, adverse effects. Repeated administration of low doses will not overwhelm the urinary system because of the rapid excretion by the kidney (half-life estimated < 24 hours). Instead a steady state concentration will be reached within three to five days of daily exposure (Gehring et al., 1973).

Erne (cited in Paulson, 1975) reported that 2,4,5-T was readily absorbed by the gut of rats, pigs, calves and chickens after oral administration. Tissue concentrations were highest in the liver, kidney, lung and spleen with half-life values ranging from 5 to 30 hours.

Human volunteers were given a single dose of either 2, 3 or 5 mg/kg of 2,4,5-T. In each of the subjects, the 2,4,5-T appeared in plasma samples one hour after administration. Maximum concentration was reached between 7-24 hours after ingestion. After 32 hours, the concentration of 2,4,5-T was detected in some urine samples. More than half of the compound was excreted in the urine during the first 48 hours, after which the excretion rate dropped markedly. The authors concluded that 2,4,5-T did not appear to undergo any metabolic transformation at the doses used in their study (Khanna and Kohli, 1975).

Gehring et al. (1973) also studied the fate of 2.4,5-T in man after oral administration. Following ingestion of 5 mg/kg, the concentration of 2,4,5-T in plasma increased rapidly and reached a peak after seven hours. The concentration then declined, with a half-life of 23 hours.

Researchers once believed that the phenoxy herbicides were excreted unchanged into the urine, but now some metabolites and conjugates are being identified. In past studies, the identity of the metabolite was not determined due to either the inability to recognize the compound or no attempt to identify it.

Concern has been voiced for those who work directly with the herbicide, such as forest sprayers, commercial applicators as well as those indirectly exposed to the herbicide from spray drift or residues. In these cases, exposure is mainly to the skin. The degree of skin absorption (percutaneous) of a chemical depends on a number of factors which all vary to some extent with the individual involved. Lavy et al. (1980) studied the extent of exposure of field workers involved in spraying operations. They concluded that workers applying 2,4,5-T with proper use, are not exposed to quantities above possible health hazard levels. Similar results were obtained in a similar study by Newton and Morris (1980).

Different areas of the body exhibit different absorption rates. Absorption from the scrotum is complete; the head and neck absorb two to six times more than the forearm (NRCC, 1978). Solvents generally increase the absorption powers of the herbi-

cides dissolved in them. Damage to the skin may also increase absorption into the body.

ACUTE AND CHRONIC TOXICITY

Toxicity of herbicides can either be acute or chronic. Acute toxicity is the rapid response of organisms to a few large doses of the chemical received over a short period of time. Chronic toxicity is the accumulation of effects resulting from exposure over a long interval (MDH, 1978).

Acute - Phenoxy acids have a low to moderate acute toxicity. The LD_{50}* values for some animals are listed in Table 1. In reviewing acute toxicity of 2,4,5-T, it is important to remember that the nature of the dose-response relationship varies with both the chemical and the organism. Individuals within a species may also vary. The formulation of the commercial product may have an influence on the toxicity but some authors concluded that differences between formulations may not be significant (Björklund and Erne, cited in Way, 1969; Dalgaard-Mikkelsen and Paulsen, 1962, NRCC, 1978). Surfactants and other additives can enhance the toxicity of the active compounds.

Signs of acute toxicity in mammals are anorexia, weight loss, depression, muscular weakness, peripheral neuropathy and posterier paralysis (Gehring and Betso, 1978).

Kociba et al. (1979) cite an unpublished sub-acute toxicity study in which no adverse effects were observed in rats fed 2,4,5-T at dose levels of 3 or 10 mg/kg/day for

*LD50 - dosage level at which 50% of the test species population is killed.

90 days. When given higher dose levels of 30 or 100 mg/kg/day, increases in liver and kidney weights were noticed. Mice involved in short-term feeding studies using samples of 2,4,5-T containing various amounts of TCDD demonstrated multiple toxic effects from treatment. The specific effects are not mentioned in the review. The dosage levels producing effects were 120 or 60 mg 2,4,5-T/kg (Highman, Gaines and Schumacher, cited in NRCC, 1978). Reports of human ingestion of 2,4,5-T indicated that essentially all of the 2,4,5-T absorbed into the body is excreted by the urine with no observable toxic effects (Piper et al., 1973; Gehring et al., 1973). Larger doses, such as found in some accidental poisoning cases or in attempted suicides can overwhelm the capacity for urinary excretion. Toxicity in these cases is dependent on the dose taken. There is no evidence to suggest the accumulation or the increasing of tissue damage with low doses (Gehring and Betso, 1978).

Chronic - Much of the data gathered on human chronic toxicity cases and 2,4,5-T deal with those people involved in either the manufacturing or the field application of the herbicide. Poland et al. (1971) reported on a follow-up health survey of 73 male employees in a 2,4-D and 2,4,5-T manufacturing plant. Less toxicity was found in organ systems such as the liver and kidneys than was reported in a similar survey of the plant six years earlier (Bleiberg et al., cited in Ott et al., 1980). Differences in results may have been due to the decrease of the level of TCDD in 2,4,5-T during those

years between studies.

Ott et al. (1980) examines the mortality experienced of a cohort of 204 employees involved in the manufacture of 2,4,5-T comparing it to the overall U.S. white male population. 75% of the men in this cohort worked for less than 12 months in jobs involving exposure. None were exposed during their entire working lifetime. Only one malignancy death was uncovered from the original cohort. This was a respiratory malignancy in a retiree who was exposed for eight years. The subject has a past history of heavy smoking.

Further human and animal chronic exposure are outlined in the following report sections.

TABLE 1 Acute Oral Toxicity of 2,4,5-T

Formulation	Species	LD50 (mg/kg/bw)	Reference
Acid	Mouse	389	Rowe & Hymas (1954)
	Rat	500	" " " "
	Guinea Pig	381	" " " "
	Chick	310	" " " "
	Dog	100	Drill & Hiratzka (1953)
Amyl Ester	Rat	750	Rowe & Hymas (1954)
Butyl Ester	Mouse	940	" " " "
	Rat	481	" " " "
	Rat	800 (in water)	Stupnikov (1972)
	Rat	275-525 (in diesel)	" "
	Guinea Pig	750	Rowe & Hymas (1954)
Isopropyl Ester	Mouse	551	" " " "
	Rat	495	" " " "
	Guinea Pig	449	" " " "

CARCINOGENICITY

The Scientific Advisory Panel to EPA believes that the carcinogenic potential of 2,4,5-T to humans is best determined in studies using samples contaminated with the dioxin TCDD rather than in studies using purified samples of 2,4,5-T. Contaminated 2,4,5-T is found in the majority of commercial products to which humans would have the greatest exposure. The following review will deal mainly with studies using contaminated 2,4,5-T. Any studies done with purified 2,4,5-T will be labelled as such.

Kociba et al. (1979) conducted a two year oncogenic study of rats administered 3, 10 or 30 mg 2,4,5-T/kg/body weight daily. The herbicide was 99% pure with no TCDD detected (detection limit 0.33 ug/kg). The tumors observed in this study are the same as historically found in this strain of rats. Statistical analysis of these data showed that the incidence of each type of tumor in any of the treated groups was comparable to that in the control group given no 2,4,5-T, with the exception of one type of tumor found in female rats given 3 mg/kg/day. This tumor showed no dose-response relationship, with the control group showing a lower than usual rate of occurrence for this type of tumor, therefore, it was not considered related to the treatment. They conclude that neither the total numbers of tumors per group, the average number per rat nor the times of observations of the tumors were affected by any levels

of treatment with 2,4,5-T.

No significant increase in tumor incidence as compared to the control group was reported by Innes et al. (1969) when two strains of mice were given the maximum tolerated dose of 21.5 mg/kg.

At the time of the Kociba et al. study, there was only one oncogenic study (published) which reports an increase in tumors due to treatment with 2,4,5-T. In that study, two strains of mice were given single dose levels of 12/mg/kg/day. One of the strains had a statistically significant increase in tumor incidence while the other strain showed no effect. In the affected strain, no specific target tissue showed the increase in tumor incidence (Muranyi-Kovaces et al., cited in Kociba et al., 1979). This contradicts the majority of oncogenic studies in which a positive response of increased tumor incidence usually affects specific target tissue(s), from which the resulting increase in tumors originates.

In 1974, Axelson and Sundell reported that among Swedish railroad workers exposed to herbicides, a significant, two-fold excess of all cancers was observed. The comparison was made to the national average. It was unclear whether the excess in cancer was due to 2,4,5-T since the workers were also exposed to 2,4-D and amitrole. At first the excess cancer was attributed to the amitrole and that exposure to the phenoxy acids had normal tumor incidence (5 cancers at all sites observed versus 2.8 expected). The authors re-examined

the data in 1977 and sorted out the effects of the amitrole exposure. They found a significant ($P<0.05$) trend indicating a clear relationship between increasing exposure to phenoxy herbicides and tumor incidence. The IARC in their review on 2,4-D concluded that the 1977 results of the Swedish study were not enough to evaluate the carcinogenicity of 2,4-D since it was used with 2,4,5-T. It would be speculation to evaluate 2,4,5-T as a carcinogen in this study because it contained the dioxin TCDD, which is a known toxic substance.

The EPA reviewed the Tung et al. (1971) report indicating an increased incidence of liver cancers among Vietnamese following the spraying of Agent Orange, a fifty-fifty mixture of 2,4-D and 2,4,5-T. According to EPA, this study "is not sufficient to be the basis of any firm conclusion concerning a causal connection between 2,4,5-T and cancer". But EPA goes on to say that this study is worth noting in view of the results which have been obtained in laboratory animals (U.S. EPA, 1979).

The Carcinogen Assessment Group (CAG) reviewed 10 chronic toxicity studies involving mice (8) and rats (2). They concluded that there is no significant evidence in the completed studies that 2,4,5-T is carcinogenic in these animals. The potential of commercial 2,4,5-T to cause cancer should not be ignored since it contains TCDD (U.S. EPA, 1979). For a summary of animal data, see Appendix 1.

It is difficult to separate the carcinogenic potential of 2,4,5-T from that of the dioxin TCDD in commercial products

used by humans. Although TCDD has been labelled as a carcinogen in laboratory animals, no report thus far has determined whether or not 2,4,5-T has any effect on the type of cancer or the severity of tumor incidence initiated by TCDD (U.S. EPA, 1979; Scientific Dispute Resolution Conference, 1979).

Van Miller et al. (1977) conducted a TCDD feeding study giving daily doses ranging from 1, 5, 50, 500 ppt and 1, 5, 50, 500 or 1000 ppb to rats. Acute toxicity was observed at the three highest dose levels (50, 500, and 1000 ppb). All animals in these groups died between the second and fourth week of the experiment. 57% of all the rats that died in the six groups fed subacute levels of TCDD had neoplastic alterations. The overall incidence of neoplasms in the six subacute groups was 38% with no neoplasms found in the 1 ppt group. The authors concluded that the high incidence of neoplasms found in the subacute doses suggests carcinogenic potential of TCDD. Because of the number of animals used and the diversity of the results, a direct link between TCDD and cancer cannot be made from the results of this study.

TCDD administered at 0.1 ug/kg/day in the diet for two years caused an increase incidence of tumors in the liver, lungs, palate and tongue. A decreased incidence of tumors in mammary glands, uterus, pituitary and pancreas compared to the normal incidence for these sites in this strain of mice was noted (Kociba, et al., cited in Leng, 1979).

Reliable reports containing a more definite conclusion on TCDD and cancer have not appeared in the literature as yet.

MUTAGENICITY

A mutagen is defined as any inherited alteration in the genetic material. For a substance to act directly as a mutagen, it must interact with DNA, directly or indirectly by affecting a component in the DNA system. The mutations create a diversity of effects. Dominant mutant genes can show up as fetal death, sterility, abnormal numbers of fingers and toes. Recessive mutations may not be expressed for several generations. Tests on bacteria, yeast cultures, plant and mammalian systems have been done to determine the mutagenic potential of the phenoxy herbicides. Submammalian testing methods have the advantage of being sensitive, giving precise information concerning the nature of the genetic change at the molecular level. Mammalian testing methods have the highest degree of relevance for man, especially if the results are positive at subtoxic levels (Fahrig, 1974).

Mutagenicity tests using bacteria have all been negative for 2,4,5-T (Zetterberg, 1978). Some of the studies were spot tests and so may not have been as sensitive as other test methods for detecting mutations.

Results from yeast tests have produced both positive and negative results. This variability may have been solved when it was discovered that yeast mutagenicity by phenoxy acid was dependent on the pH in which the yeast cultures were treated. Zetterberg (1978) notes that some of the experiments obtaining negative results using yeast cells

were carried out under neutral pH (pH - 7). He theorized that the cells were unable to take up the dissociated form of the herbicides and high doses would not be mutagenic at neutral pH. Since 2,4,5-T is a weak acid, at a lower pH a greater percentage of the compound is in the undissociated form and may more easily enter the cells. Zetterberg's own experiments with yeast cells revealed 2,4,5-T as toxic and mutagenic when the pH of the treatment solution is lower than 4.5.

The sex-linked recessive lethal test system is a more reliable measurement of the mutagenic potential of phenoxy acids. Rasmuson and Svahlin (1978) tested for mutagenicity in Drosophila melanogaster using a sex-linked, genetically unstable system. There was no significant increase in the frequency of somatic mutations when the strains of flies were exposed to 2,4,5-T. An increased number of recessive lethals were observed in Drosophila by Majumdar and Golia (cited in Ramel, 1978) after high dose treatment with 2,4,5-T. Ramel (1978) reviewed several studies of mutagenicity in Drosophila and from available data concluded that 2,4,5-T has only weak mutagenic effects in animals, according to recessive lethal tests.

Several mutagenicity tests on mammals have been done, mainly with mice, rats and gerbils. Chromosomal damage in the form of chromatid aberrations or chromosome adhesion was observed in single doses above 0.001 mg/kg/body weight of 2,4,5-T given to rats (Yefimenko, cited in NRCC, 1978). Majumdar and Hall (cited in Ramel, 1978) investigated the

effect of 2,4,5-T on chromosomes from bone marrow cells of the Mongolian gerbil. The animals received five daily injections of 2,4,5-T which contained no measurable amounts of dioxin. An increase of chromosomal aberrations such as gaps, breaks and fragments were found at and exceeding 50 mg/kg/day. Some controversy exists concerning the authors classification of aberrations and interpretation of the results, rendering the study inconclusive.

A study was done on industrial workers who had been exposed to 2,4,5-T on a regular basis for as long as three years and greater. After a complete medical study, no meaningful differences were found between the control group, having no contact with 2,4,5-T, and the exposed group. Chromosomes of the exposed workers were normal (Johnson, 1971).

Tung et al. (cited in National Academy of Science, 1971) reported chromosomal abnormalities in Vietnam after exposure to the herbicides used in Agent Orange. The National Academy of Science determined Tung's study as inadequate. No attempt was made to distinguish herbicide exposure from other agents which are known to produce similar chromosomal aberrations.

Presently, investigations of chromosomal aberrations induced by the phenoxy acids in mammals are inconclusive. For that reason, reports of negative results in mammalian test systems should not be interpreted as no possible mutagenic effects.

Direct assessment of the mutagenicity of TCDD is rather limited based on published reports according to a 1979 review

prepared by the Scientific Dispute Resolution Conference (See Appendix 2).

Hussain et al. (cited in Moore, 1978) carried out several experiments using bacterial systems. Results were strain-related for Salmonella typhimurium, reporting both positive and negative findings for increased mutation frequencies. One of the strains tested indicates that TCDD acts as a frame shift mutation for that strain.

Chromosomal analysis of people who were exposed to TCDD in Seveso, Italy revealed an increase in gaps, breaks and rearrangement of chromosomes in somatic cells. These results were preliminary and based on a small number of samples (U.S. EPA, 1978).

Cytogenetic evaluations of TCDD in male rats found no evidence of chromosomal aberration in the bone marrow cells in treated animals. Rats were given doses up to 20 ug/kg/day for five days orally or through the lining of the abdomen (Green and Moreland, cited in NRCC, 1978).

Further evidence and testing is needed to conclusively label TCDD as a mutagen. As with 2,4,5-T, the results from negative or inclusive studies should not be ignored. Errors in testing or inappropriate testing methods may account for some of the negative results obtained.

TERATOGENICITY/EMBRYOTOXICITY

The Minnesota Department of Health defines teratogenic as a developmental disturbance of the embryo, resulting in congenital malformations. They define embryotoxic as any harmful effect on the embryo or fetus. EPA states that feto or embryotoxicity includes teratogenicity (U.S. EPA, 1980). Common examples of fetotoxicity are reduced fetal weight, delayed growth and maturation, increased fetal abortions, reabsorptions, and stillbirths. Teratogenicity includes grossly observable birth defects such as cleft palate, club foot and exemcephaly; also internal defects such as extra ribs or changes in kidney structure. The use of these two terms is not always consistent and there exists an annoying problem of semantics. For example, what Dow Chemical Company considers embryotoxic, other groups consider teratogenic, or there may be no discrimination between the two terms at all. Interpreting studies and reviews on teratogenicity and fetotoxicity must be done carefully, keeping in mind possible term discrepencies. Generally, the studies describe possible adverse effects on the developing fetus. Reference to the abnormality found and what the investigator labels it will be noted in the following review.

Rats - Rats administered 2,4,5-T (30 ppm TCDD) at a dose of 4, 6, 10 or 46.4 mg/kg on days 10 through 15 of gestation produced a significant increase in fetal mortality. The two lower doses produced a significant increase in abnormal

fetuses, particularly cystic kidneys (Courtney et al., 1970).

Rats were given 2,4,5-T in a single daily dose at various dose levels ranging from 25 - 300 mg/kg. Teratologic potential was demonstrated at or above 100 mg/kg, with an increased incidence of skeletal anomalies on days 6 - 15 of gestation and an increase in fetal deaths. Results from the study showed a significant dose-related effect with the average incidence of affected fetuses moving from less than 10% in the controls to more than 50% at the 150 mg/kg dose level (Khera and McKinley, 1972).

Mice - Two strains of mice were fed 2,4,5-T which contained approximately 30 ppm of the contaminant TCDD. Doses were administered either orally or subcutaneously at a rate of 4 - 113 mg/kg/body weight for each mouse during specified periods of gestation. Administration of 2,4,5-T to strain A on days 6 through 14 produced significant increases in the percentage of abnormal litters and abnormal fetuses per litter. Anomalies produced were almost exclusively cystic kidney and cleft palate. No difference was noted due to route of administration. Strain B mice showed a significant increase in fetal mortality and cleft palate when administered 113 mg/kg in honey. 2,4,5-T did not produce cystic kidneys in this strain (Courtney et al., 1970).

Bage et al. (1973) studied the effects on mice of two commercial phenoxyacid formulations in Sweden. One formulation contained 2,4-D and 2,4,5-T in a 2:1 mixture and the other formulation contained only 2,4,5-T. Both were given at a

dose level of either 110 mg/kg or 50 mg/kg. Fetal mortality was significantly increased at both dose levels of 2,4,5-T and at the high dose level (100 mg/kg) of the mixture compound. These same dosage groups produced an increase in frequency of cleft palate. Fetal weight was reduced in the higher dosage levels. These low-weight fetuses often showed retarded skeletal development as well. The degree of skeletal malformations was most severe in the higher dose of 2,4,5-T only. Cystic kidneys were not found. The authors conclude that high doses of phenoxyacetic acids are teratogenic, causing fetal death and retarded fetal growth in mice. The 2,4,5-T sample contained the contaminant TCDD at a level less than 1 ppm. The mixture compound was also contaminated as it contained the 2,4,5-T.

Gaines et al. (cited in NRCC, 1978) reported on a study using pregnant mice of four different strains and technical grade 2,4,5-T. Administration was by stomach tube on days 6 - 14 of pregnancy. In one of the strains, the 15 mg/kg/body weight/day dose level proved teratogenic, producing cleft palate. In another strain, 30 mg/kg/body weight/day had the same effects. No level of TCDD was stated.

Rabbits - In rabbits orally dosed with 40 mg/kg/body weight/day of 2,4,5-T (containing approximately 0.5 mg/kg TCDD) on days 6 - 18 of gestation, there was no indication of teratogenicity, embryotoxicity or maternal toxicity (Emerson, cited in Johnson, 1971).

Hamsters - The Advisory Committee on 2,4,5-T to EPA reviewed

a study by Collins and Williams concerning the oral administration of 2,4,5-T to golden hamsters (1971). The hamsters were treated on day 6 - 10 of gestation with 20 - 100 mg/kg daily. Some of the samples contained the dioxin TCDD. Fetal mortality was greatly increased by the 2,4,5-T samples containing TCDD. Mortality was also high and dose-related in those animals given 2,4,5-T containing no detectable TCDD. No malformations were produced by 2,4,5-T alone below the 100 mg/kg dose whereas all dosages of the TCDD-containing 2,4,5-T produced malformations. The original study had some apparent discrepancies concerning the calculation of the malformation rates.

Humans - There are several reported incidences involving pregnant women and exposure to phenoxy herbicides, mainly 2,4,5-T. The most noted come from Sweden, Vietnam, Sevesto, Italy and most recently Alsea, Oregon.

During 1970, reports of miscarriages and the births of malformed children in Sweden drew national attention. The mothers believed they had been exposed to phenoxy herbicides used in defoliation projects. The cases were investigated by a group of experts appointed by the Swedish Poisons and Pesticide Board. A causal relationship between the mothers' exposure to 2,4,5-T and birth defects and abortions could not be established (Giftnamnden, cited in Backstrom, 1978).

Surveys in Vietnam concerning possible teratogenic effects due to the spraying of Agent Orange are somewhat incomplete. The EPA Advisory committee on 2,4,5-T could not

find evidence for any marked increase in the number of birth defects in the areas sprayed (Advisory Committee, 1971). Any increase in the number of reported cases of human birth defects may have been due to better collection methods for data compared to that of earlier years. But, as stated earlier, no increase in birth defects was found. Problems also arose in locating the areas where the mothers lived during possible exposure periods due to the transient habits of the inhabitants during the war. In their 1974 report, the National Academy of Sciences could find no evidence of an increase in congenital malformations related to herbicide spraying while investigating hospital records in Vietnam. EPA concluded that the reporting of cases in Vietnam "was and still is peacemeal" (U.S. EPA, 1979).

Seveso, Italy was an incident for which reports are inconclusive. In July of 1976, a reactor in the ICMESA plant exploded and exposed products including TCDD. The ICMESA plant manufactures, among other substances, trichlorophenol. Concern arose over the possible exposure to a large number of women living in the area who were pregnant at the time of the explosion.

An epidemiological study was carried out and the contaminated area was divided into Zone A and Zone B, each having a calculated concentration of dioxin of approximately 20 ug/m^2 and 4 ug/m^2 respectively. In the two zones, 623 pregnant women, of which a third were in their first trimester, were identified (Tuchmann-Dupleisis, 1978). A comparison of the

epidemiological data shows no significant change in the frequency of spontaneous abortions after the Seveso incident (Homberger et al., 1977). The examination of 30 embryos produced by therapeutic abortions and four spontaneous abortions did not reveal any pathological malformations (Gropp, cited in Tuchmann-Dupleisis, 1977).

The U. S. EPA (1979) acknowledges the negative results of the reproductive epidemiology study as not providing definite evidence of increased risk of spontaneous abortions or congenital malformations following the explosion in Seveso. The Agency goes on to say that the negative results do not provide evidence for the absence of teratogenic risk in humans due to the incomplete data available and inadequate evaluation of that data.

The most current epidemiology study in the literature is the Alsea II report. This report was a major factor in EPA's decision to initiate RPAR proceedings on 2,4,5-T. On February 28, 1979, the Federal EPA took emergency action to halt the spraying of 2,4,5-T. Emergency suspension of the herbicide indicates that there may be a possible imminent threat to human health. The suspension was based on EPA's findings from the Alsea II study and also reports to adverse health effects in laboratory animals (U.S. EPA, 1979). The Alsea II study has generated much criticism from the scientific community and caused the public much concern. Because of its relative importance, details of the study conclusions along with the related criticism will be outlined.

During 1978, the EPA issued a notice of Rebuttable Presumption Against Registration (RPAR) for all pesticide products containing 2,4,5-T. EPA issues RPAR notices to initiate a comprehenisve public review of all 2,4,5-T registrations and all pending applications for registration of 2,4,5-T products. In response to this notice, a group of eight women in Oregon wrote to EPA relating their experiences of excessive abortions during the period of 1972-1977. They believed that their miscarriages were directly related to the spraying of 2,4,5-T in a nearby forest.

The initial investigation into the Oregon claims (Alsea I) turned up no positive correlation between the women's exposure to 2,4,5-T and the miscarriages. The EPA then decided to perform an epidemiological study of the area to determine if the occurrence of the spontaneous abortions during the period of 1972-1977 had any relation to 2,4,5-T. Information and data from hospitals on the occurrence of spontaneous abortions in the Alsea Study area was compared to similar data from a Rural area (control area) which had little or no known use of 2,4,5-T. Data from an Urban area (Corvalis) near Alsea was also collected. Spray patterns and herbicide usage were evaluated. After statistical analyses of the spontaneous abortions and spray data of the three areas, EPA reached three general conclusions.

1. EPA - "The 1972-1977 abortion rate index for the study area is significantly higher than those for either the Control (rural) or the Urban area".

The available reviews all agree that EPA failed to take into account the differences in medical practices of the three areas. In the Urban area, clinics also treat spontaneous abortion cases. Any woman who went to a clinic for treatment would not be included in the abortion data for the Urban area as only hospitalized spontaneous abortions were included. The number of hospitalized spontaneous abortions (HSA) were used to calculate the HSA Index. This index was the EPA's measure of spontaneous abortions, defined as 20 weeks or less gestation, in each of the three areas. Failure to count clinical cases reduced the HSA Index for the Urban area. Mantel (1979) points out that the difference between abortion rate index for the Study area (8.1%) versus the Control area (6.5%) is statistically insignificant (P=0.115). The difference is statistically significant though between the study area and the Urban area (4.4%). As already pointed out, the Urban data is incomplete and cannot be comparitively used. EPA recognized this shortcoming but uses the comparison anyway.

2. EPA - "There is a statistically significant seasonal cycle in the abortion index in each of the areas with a period of about four months. In particular, there is an outstanding peak in June in the Study area."

The raw data on abortion rates month by month for the six years is not found in the published Alsea II report, so it is difficult to establish the peak monthly HSA Index for any one year. According to Wagner et al. (1979) who did examine the raw data in the study area, the peak monthly

HSA Index for the year occurred in five different months during the six year study period. June had the peak index in 1976 only. It is incorrect for EPA to present this peak as a repetitive event. The June peak consisted of 1.4 in excess of the usual number of abortions reported.

3. EPA - "There is a statistically significant cross correlation between the Study area spontaneous abortion index and the spray patterns in terms of pounds applied by months in the Alsea Basin, 1972-1977, after a lag time of two or three months." These results were based on the six year aggregate of abortion and spray data and are confirmed by the analysis of the two 3-year aggregates.

The spray data collected by EPA is incomplete and non-representative of the Study area. Data collected represents only a small percentage of the actual use during the six-year period. Spray data for the first three years was not as complete as data for the final three years, indicating the alleged increase of use in 1975-1977 was actually due to better collection methods. Actual amount of 2,4,5-T sprayed in the spring was 39,621 pounds in 1972-1974 and 45,555 pounds in 1975-1977 (Wagner et al., 1979). Reviewers feel there is no statistically significant cross correlation between the Study area and the spontaneous abortion index. 1976 appeared to be the only year spontaneous abortions peaked in June and usage figures cite 1973 as the year of peak use of 2,4,5-T during the spring. The 1973 spontaneous abortion rate peak did not occur in June.

Below is a listing given by Witt (1979) of sources for evaluation of the Alsea II Report. The reader should consult these reports, as well as the EPA's Alsea II Report for more detailed information and explanations.

Lamm, S. H., Tabershaw Associates, August 6, 1979.

Downs, T., University of Texas, School of Public Health, March 21, 1979.

Mantel, N., George Washington University, March 26, 1979.

Wagner, S. L. et al., Oregon State University, Environmental Sciences Center, October 25, 1979.

Blau, G. E. et al., DOW Chemical Company, March 4, 1979.

Bedford, D. M. O. et al., Division of Public Health, Wellington, New Zealand, May, 1979.

Smith, A. H., Wellington Clinical School of Medicine, Wellington, New Zealand, May, 1979.

TCDD

TCDD is a specific teratogen which interferes with only a few special developmental processes. These are cleft palate and kidney abnormalities of a special type (Neubert et al., 1973). Also, the teratogenic effectiveness of TCDD is highly dependent on time of administration. On one day of gestation the effect may be greater than on the day before or the day after. Considerable variation in sensitivity to the teratogenesis by TCDD can be found among different strains of an animal species, such as mice.

The majority of teratogenic studies published use rats and mice as test subjects. Treatment with TCDD is usually daily throughout the period of organogenesis, which for these

species is between days 6 - 15 of gestation. Because TCDD is highly toxic, most dosages are at the microgram level (ug). (1 ug equals .001 mg)

Rats given 0.1 ug TCDD/kg daily exhibited impairment of reproduction with significant decreases observed in fertility, litter size, gestation survival and newborn body weight. No adverse effect on reproduction was noted among rats given 0.001 ug TCDD/kg daily (Murray et al., 1979). Subcutaneous treatments of TCDD to rats did not produce malformations or excessive fetal mortality at doses of 0.5 ug/kg daily or 2 ug/kg daily although possible kidney anomalies were noted. The EPA Advisory Committee concluded that TCDD, given to pregnant rats, caused embryolethality and occasional teratogenicity at doses below the maternal toxic level (1971).

In mice, the highest degree of malformations can be produced when the treatments are given on the 11th day of gestation (Neubert et al., 1973). Mice dosed orally with 0.3, 3.0, 4.5, or 9 ug/kg of TCDD on days 6 - 15 of gestation exhibited a significant increase in the incidence of cleft palate at all dosage levels and was dose-related (Neubert and Dillman, cited in Moore, 1978). Moore et al. (1973) found the effects of TCDD on the incidence of cleft palate in mice to be dose- and time-related. A dose-related increase of kidney anomalies were also observed. Dosage levels were 1 or 3 ug/kg on days 10 - 13 of gestation.

TCDD is capable of triggering malformations of certain types at repeated or even single doses of as little as 1 - 10

ug/kg (Neubert et al., 1973). It is one if not the most effective teratogen known.

The role of TCDD as a teratogen in other laboratory species is yet unclear.

Soil - TCDD is not mobile in soils. Upon application, it generally remains on or near the soil surface and is not leached into the soil even after rainfall and irrigation (Helling et al., 1973). Degradation of TCDD, as it occurs in formulated products, is mainly by sunlight (Crosby and Wong, 1977; Matsumura and Benezet, 1973). Pure TCDD is not photodegraded which led Crosby and Wong to the conclusion that the presence of a solvent or pesticide which can act as an organic hydrogen-donor is necessary for the photodegradation of TCDD.

TCDD that does become incorporated into the soil appears to be fairly persistent as compared to other organic compounds. This is consistent with its physical properties of being insoluble and nonpolar as well as having no readily metabolizable groups (Helling et al., 1973). Matsumura and Benezet (1973) found only 5 out of 100 microbial strains known for their ability to degrade persistent pesticides able to degrade TCDD. Kearney et al. (1972) estimates the half-life of TCDD to be one year. Soil uptake of TCDD by plants is highly unlikely (Isensee and Jones, cited in Helling et al., 1973).

Water - In natural waters, naturally occurring dissolved organic compounds and the organic solvents used in formulations of the phenoxy herbicides may serve as effective hydrogen donors. TCDD, in the pure form, is insoluble in water - 0.2 ppb (Helling et al., 1973). It would then form a thin film on the water surface which can be degraded by the sunlight.

Otherwise, TCDD would degrade slowly in the water (USDA et al., 1979).

Mammals - TCDD appears to be absorbed fairly readily by mammals (NRCC, 1978). Fries and Marrow (1975) calculated the assimilation efficiency to be 50-60%. Piper et al. (1973) found the absorbed TCDD is localized in the liver and fat tissues at a level which is approximately ten fold that in other tissues. The liver and fat are the two major body compartments for TCDD storage in the body. Almost 30% of the single oral dose given to the rats by Piper et al. was eliminated via the feces during the first 48 hours following treatment. In studies where rats were given more that a single dose, elimination was also via the feces with an estimated half-life of 12 - 24 days (Moore, 1978).

The question that is asked frequently is: will the TCDD residues bioaccumulate? Samples of milk were collected from cows grazing on areas treated with normal applications of 2,4,5-T and analyzed for TCDD residues. At a detection limit of 1 ppt, control samples were identical to those samples from the treated area. No TCDD was found (Mahle et al., 1977).

Specimens of fat taken from steers allowed to graze on 2,4,5-T treated rangeland showed no response for TCDD. When the steers were confined to a fenced pasture completely sprayed with 2,4,5-T ten days earlier, three of the seven animals exposed gave a positive response at extremely low levels of 3 to 4 ppt of TCDD (Kocher et al., 1978). In their assessment

report on 2,4,5-T, the EPA RPAR Assessment Team reviewed the residue studies done on animals. The authors state that residues in animal samples from areas where 2,4,5-T is used at normal rates tend to show little, if any, bioaccumulation of TCDD. Bioaccumulation is possible though.

In the April 23, 1980 issue of Pesticide and Toxic Chemical News, new Dow Chemical studies were revealed which show accumulation of TCDD does occur in cattle and sheep, and that TCDD residues appear in the milk and cream of cows. One study concluded that the half-life of TCDD in beef fat was 15-18 weeks. Exposure of the cattle to TCDD in this study was considerably higher that what the animals would normally incur. TCDD residues in milk of cows fed 5-500 ppt of TCDD for two or three weeks ranged from non-detectable at 5 ppt of TCDD to 89 ppt at dose levels of 500 ppt of TCDD. TCDD residues found in cream were about ten times larger than those found in the milk.

The opinion is split in the literature concerning the bioaccumulation of TCDD in the environment. Most authors do agree that a reduced content of TCDD in 2,4,5-T would be an added safety feature for the future use of the herbicide until more facts are known.

APPENDIX I. Summary of Animal Data Available to Assess Potential Oncogenicity of 2,4,5-T.

Species	Strain	Route Administration	Results	References
Mouse	F1 hybrid of C57B1/6 and C3H/Awf (Strain "X" and "A")	Diet	No increase in tumors due to ingestion of 2,4,5-T	Innes et al., J.N.C.I., 1969
Mouse	F1 hybrid of C57B1/6 and AKR (Strain "Y" or "B")	Diet	No increase in tumors due to ingestion of 2,4,5-T	Innes et al., J.N.C.I., 1969
Mouse	Strain "X" or "A" of above	Subcutaneous Injection	No increase in tumors due to injection of 2,4,5-T	Bionetics Res. Laboratories' report to N.C.I., Aug., 1968
Mouse	Strain "Y" or "B" of above	Subcutaneous Injection	No increase in tumors due to injection of 2,4,5-T	Bionetics Res. Laboratories' report to N.C.I., Aug.. 1968
Mouse	XVII/C	Diet	No increase in tumors due to ingestion of 2,4,5-T	Muranyi-Kovacs et al., Brit. J. Cancer, 1976
Mouse	C3Hf	Diet	Statistical increase in overall incidence of tumors *	Muranyi-Kovacs et al., Brit. J. Cancer, 1976
Mouse	XVII/C	Subcutaneous Injection	No increase in tumors due to injection of 2,4,5-T	Muranyi-Kovacs et al., Europ. Assoc. Cancer Res., 1977
Mouse	C3Hf	Subcutaneous Injection	No increase in tumors due to injection of 2,4,5-T	Muranyi-Kovacs et al., Europ. Assoc. Cancer Res., 1977
Rat	Sprague-Dawley	Diet	No increase in tumors after lifetime ingestion of 2,4,5-T	Kociba et al., 1979
Rat	Sprague-Dawley	Diet	No increase in tumors based on preliminary data available at this time	Status Report - Laboratory for Pharmacology and Toxicology, Hamburg, Germany, 1978

* Attempts to validate this study by EPA have been unsuccessful (EPA Carcinogenic Assessment Group Statement of February 23, 1979: "In one of several studies conducted by Muranyi-Kovacs, an oral study at a single dose of 80 ppm in the diet, 2,4,5-T induced a statistically significant ($p<0.03$) increase of all tumors combined in female mice as compared to the controls. To discuss the design, execution and interpretation of the study, the CAG has communicated with the principal author at the Curie Foundation, Marsville, France. The discussion brought out the following points: 1) the animal husbandry was inadequate; 2) the dose used, 80 ppm, was only 1/40 of the LD50 and it appears to be less then maximum tolerated dose; 3) some mice were arbitrarily excluded from the calculations of tumor incidence; 4) the histology data on all animals were not available; 5) the author recommended that more adequate studies be conducted in a greater number of species.")

Source: Scientific Dispute Resolution Conference of 2,4,5-T, 1979. pp. 41, 42.

APPENDIX II. Summary of Mutagenesis Tests with 2,4,5-T.

Species and Type of Test	References	Results
MICROORGANISMS		
Salmonella typhimurium	Anderson, et al. 1972. J. Agr. Fd. Chem. 20:649; Ercegovich and Rashid. 1977. 174th ACS Meeting	Negative
Saccharomyces cerevesiae	Siebert and Lemperle. 1974. Mut. Res. 22:111; Fahrig. 1974. IARC Publ. 10, 161	Negative
Bacillus subtilis	Shirasu, et al. 1976. Mut. Res. 40:19	Negative
Senatia marcesrens and Escherichia coli	Fahrig. 1974. IARC Publ. 10, 161	Negative
DROSOPHILA		
Sex-linked recessive lethals	Majamdur and Golia. 1974. Can. J. Genet. Cytol. 16:465	Slight Increase
Sex-linked recessive lethals	Vogel and Chandler. 1974 Experientia 30:621	No Increase
Somatic cell mutations	Rasmusen and Svahlin. 1978. Ecol. Bull. 27:190	No Increase
MAMMALIAN SYSTEMS		
Mice dominant lethal and micronucleus	Buselmaier, et al. 19721 Biol. Zbl. 91:311; Jenssen and Renberg. 1976. Chem. Biol. Interact. 14:29	Negative

Appendix II (Cont'd)

Species and Type of Test	References	Results
MAMMALIAN SYSTEMS (Cont'd)		
Cytogenetic effects - gerbils	Majamdur and Hall. 1973. J. Hered 64:213	Inconclusive
Cytogenetic effects - mice	Davring and Hultgren. 1977. Hereditas 85:123	Inconclusive
HUMAN DATA		
Cytogenetics - workers	Kilian. 1975. NYAS Workshop, March 28; Johnson. 1971. Biosci. 21:899	Negative

Source: Scientific Dispute Resolution Conference on 2,4,5-T, 1979. pp. 45, 46.

APPENDIX III. Summary of Mutagenesis Tests with TCDD.

Species and Type of Test	References	Results
MICROORGANISMS		
Escherichia coli	Hussain, *et al.* 1972. Ambio 1:32	Increased Mutations
Salmonella typhimurium TA-1530	Hussain, *et al.* 1972. Ambio 1:32	Negative
Salmonella typhimurium TA-1532	Hussain, *et al.* 1972. Ambio 1:32	Increased Mutations
Salmonella typhimurium TA-1532	Selier. 1972. Experientia 29:622	Increased Mutations
Salmonella Tester Strains	Communication of B. Ames to USDA March, 1974	Negative
MAMMALIAN SYSTEMS		
Dominant lethal, rats	Khera and Ruddick. 1973. Adv. Chem Series 120	Negative
In vivo cytogenetics, rats	Green, 1975. Unpublished Report	Negative
HUMAN DATA		
Seveso plant workers, cytogenetics	Rohrborn, *et al.* 1978. Mut. Res., in press	Inconclusive
Seveso, fetal - fetal material, cytogenetics	Reggiani. 1978. Arch. Toxicol. 40:161	Inconclusive

SOURCE: Scientific Dispute Resolution Conference on 2,4,5-T, 1979. p. 47.

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